REMARKS

Reconsideration is requested.

Claims 1-21 are pending.

The Section 112, second paragraph, rejection of claims 1-16 and 18-19 is obviated by the above amendments. Reconsideration and withdrawal of the rejection are requested.

The Section 102 rejection of claims 1, 2, 4 and 6 over Meucci (U.S. Patent No. 5,135,875) is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing comments.

According to the examiner, Meucci teaches:

"a method of testing for hydrophobic drugs in patient samples by precipitating with a mixture of zinc sulfate, alcohol and acid, centrifuging and analyzing the supernatant (...)" See, page 3 of the Office Action dated December 21, 2001 (Paper No. 8).

The applicants submit however that Meucci et al described a specific reagent to be used in analytical systems to determine the presence of hydrophobic analytes in biological test sample by an immunoassay.

The improvement of Meucci's invention aims to avoid protein denaturation, used as a reagent of the immunoassay, by adding glycol, glycerol or a combination thereof during the interferent protein precipitation. According to Meucci et al, the specific reagent is:

"The precipitation reagent of the present invention [i.e., of Meucci] comprises from between about 5.0 mM and about 100.0mM of a ***zinc salt****, from between about 30% (w/v) and about 100% (w/v) of* ***a straight or branched chain alcohol having from 1 to 4 carbon atoms****, and from between about 5% (w/v) and about 50% (w/v) of* ***a glycol****. The precipitation reagent may further comprise from between about 0% (w/v) and about 20 (w/v) of* ***an acid****."* See, Meucci, column 2, lines 3-11 (emphasis added).

According to Meucci et al, the zinc salt component is selected from the group consisting of zinc sulfate, zinc choride, zinc acetate, and the like. See, column 2, lines 40-43 of Meucci. In Meucci et al. the alcohol component of the precipitation reagent is selected from the group consisting of methanol, ethanol, propanol, butanol, and mixtures thereof. See, column 2, lines 49-51 of Meucci. Moreover Meucci et al describe their glycol component of the precipitation reagent as being selected from the group consisting of ethylene glycol, propylene glycol, polyethylene glycol, polypropylene glycol, glycerol and the like. See, column 2, lines 55-58 of Meucci. Meucci et al teach that in addition to the other components (zinc salt, alcohol and glycol) an acid component, such as 5-sulfosalicylic acid, trichloroacetic acid, hydrochloric acid, acetic acid and the like, may be used. See, column 2, lines 65-68 of Meucci.

In one embodiment of the presently claimed invention, the reagent comprises: zinc sulfate; a solvent component which is selected from acetonitrile/2-propanol (1:1), benzene, toluene, dichloromethane, chloroform or its mixtures (nonpolar solvents) as well as water, alcohols or its mixtures (polar solvents); and, an acid component which is the ascorbic acid. The following table summarizes the comparison of Meucci and this embodiment of the present invention.

REAGENT	
Meucci et al	Present Invention
Zinc sulfate	Zinc sulfate
Alcohol: methanol, ethanol, propanol, butanol, and mixtures thereof.	Nonpolar Solvent: acetonitrile/2-propanol, benzene, toluene, dichloromethane, chloroform, or its mixtures. Polar Solvents: water, alcohols or its mixtures
Glycol, glycerol	-
Acid: 5-sulfosalicylic acid, trichloroacetic acid, hydrochloric acid, acetic acid, and the like	Acid: ascorbic acid

The alcohol component in Meucci et al participates in maintaining the hydrophobic analyte in solution, and precipitates proteins and conjugated proteins as

well. According to the present invention the alcohol is included to extract the analyte from the test sample. This purpose is different from that of Meucci et al, wherein the alcohol is not used as a solvent. In Meucci et al a glycol component, including glycerol, is used. Meucci states that the glycol component "*serves to decrease the toxicity of the other components of the precipitation reagent, particularly the alcohol component, and stabilizes cellular receptors and specific binding proteins which are employed in an assay system, particularly antibodies, by preserving the binding integrity thereof.*" See, Meucci, column 2, lines 58-64.

The present invention does not use a glycol component, as there is no toxicity to be decreased. This is because the alcohol (as well as the other components) of the present invention is used as a solvent, and as such it is used to extract the analytes which is different from the purpose described by Meucci et al.

In addition to the difference between the group of components, showed in table above, it is noted that the acid component used by Meucci serves to precipitate and denature the interfering proteins since according to the method used by Meucci this is a very important feature. In the present invention, the acid component is used as an antioxidizing agent as, in the present invention, there is no problem with interfering proteins. This is the reason why the acids are chosen from different groups. That is, the acid described by Meucci and the acid used in the present invention have different purposes. Additionally, the acid in the patent of Meucci is an optional ingredient.

Withdrawal of the Section 102 rejection is requested.

The Section 103 rejection of claims 3 and 7-10 over Meucci is traversed. Reconsideration and withdrawal of the rejections are requested in view of the above and the following.

The Examiner is requested to indicate, with particularity, where the cited art provides motivation to use a stronger concentration of zinc sulfate in a smaller added volume to perform the same results as in the presently claimed invention. Meucci et al did not describe the concentration of the components, so the applicants believe one

skilled in the art would not have achieved an optimal condition using the teachings from Meucci for the concentration of zinc sulfate.

With reference to the present invention, the applicants submit that in addition to the optimal concentration of the components of the present invention as well as the specific assays to measure the drug concentration, neither of which are described or suggested by Meucci et al, the alcohol and acid components of the present invention were selected from a different group from that selected by Meucci et al. Thus, it would not have been obvious to one skilled in the art to use the teachings from Meucci et al regarding the components, as the components described by Meucci et al have different purposes from those as described in the present invention. Such a different purpose is evidence of a lack of motivation in the art to make the claimed invention. As mentioned by the Examiner, Meucci et al fail to teach the concentration or assays claimed in the present invention. In the present invention, monitoring patient compliance and bioavailability of drugs contained in body fluids must be determined to follow up the efficacy of treatment. In fact, sometimes the use of glycol, glycerol or their mixtures masks the results and monitoring patient compliance is not possible.

The claimed invention is submitted to be patentable over Meucci and withdrawal of the Section 103 rejection over the same is requested.

The Section 103 rejection of claims 5 and 11-21 over Meucci in view of Lam (Journal of Liquid Chromatography, 12(10), 1851-1872 (1989)), is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing comments.

The micro – scale protein precipitation procedure of Lam et al is applicable to the HPLC of drugs in serum at minimum detection levels of 0.5 mg/ml and 0.1 mg/ml by ultra-violet and fluorescence detection, respectively. The protein precipitation occurs prior to testing. Its first step is to mix 10 ml of 10% w/v of zinc sulphate solution to 100ml of the patient sample or serum standard. The serum specimen turns milky but protein precipitation is not complete. Then 100ml of methanol or acetonitrile, depending on the solubility of the drug in the respective solvent, is added and followed by vortexing.

The method of the present invention utilizes reagents, in different concentrations as demonstrated in the examples, and obtains drug detection in body fluids down to 0.3μg/ml. This demonstrates that the applicants' method is more efficient in the extraction of the drug from the body fluid and, consequently provides improved drug monitoring. The applicants method allows one to efficiently strip off the drug which became bound to proteins contained in the biological fluid, mixing to this fluid the adequate drug solvent with the other reagents of the applicants' method.

In the Lam et al method, when the solvent is added, the precipitation has already begun (the serum specimen turns milky) so, part of the drug has become bound to the precipitate protein.

Moreover, the applicants' method can be used to detect the drug concentration in small quantities of body fluids, like saliva, and the method permits the processing of many samples in a simultaneous fashion (about 100 samples in one hour). In addition, the method may be used to measure hydrophobic or non-hydrophobic drugs. These advantages of the presently claimed invention were not obvious in view of the cited art.

Furthermore, Elsbach teaches that rifampicin is hydrophobic and Bergqvust teaches first to use the zinc sulfate and after the acetonitrile to precipitate proteins in a method for simultaneous determining of antimalarial drugs. The above considerations clearly show that the applicants' method presents an inventive step and would not have been considered obvious by conjugating the use of acetonitrile proposed by Lam et al, with the alcohol by Meucci et al to precipitate proteins with zinc sulfate as taught by Lam et al.

The claims are submitted to be patentable over the cited art and withdrawal of the Section 103 rejection over Meucci and Lam is requested.

In view of the above, the claims are submitted to be in condition for allowance and a Notice to that affect is requested.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____
B. J. Sadoff
Reg. No. 36,663

BJS:plb
1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS

1. (Amended) Method of monitoring patient compliance and bioavailability of drugs contained in body fluids comprising the following steps:

(a) mixing and shaking mechanically the body fluid with aqueous zinc sulfate solution, an appropriate solvent, and optionally an antioxidizing agent to precipitate proteins and strip off bound drug;

(b) [centrifugating] centrifuging the mixture to obtain the separation of phases;

(c) recovering the supernatant and measuring the drug concentration [proceed to drug concentration measurement].

2. (Amended) Method according claim 1 wherein the concentration of the aqueous zinc sulfate solution ranges [varies] from 0.1 M to 5.0 M.

3. (Amended) Method according claim 2 [3]wherein the concentration of the aqueous zinc sulfate solution ranges from 0.2 M to 1.0 M.

10. (Amended) Method according claim 1 wherein the drug to be analyzed is selected from the group of antimonials, itraconazole, [and] proteinase inhibitors [or the] and reverse transcriptase inhibitors.

11. (Amended) Method of monitoring patient compliance and bioavailability of rifampicin contained in body fluids comprising the following steps:

(a) mixing and shaking mechanically the body fluid with aqueous zinc sulfate solution, an organic solvent selected from the group consisting of acetonitrile / 2-propanol (1:1), benzene, toluene, dichloromethane, chloroform or mixtures thereof and[,] optionally an antioxidizing agent to precipitate proteins and strip off bound drug;

(b) centrifuging the mixture to obtain the separation of phases;

(c) recovering the [organic phase] supernatant and [proceed to drug concentration measurement by] measuring the drug concentration by using a colorimetric assay or a High-Performance Liquid Chromatography method.